BLOOD CHEMISTRY AND MORPHOMETRIC COMPARISONS BETWEEN HARBOR SEAL PUPS FROM TUGIDAK ISLAND AND WITHIN PRINCE WILLIAM SOUND, ALASKA: USING CLUSTER ANALYSIS TO ASSESS HEALTH STATUS

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INTRODUCTION

Populations of marine mammals and seabirds in the Gulf of Alaska and Bering Sea have experienced major declines over the past two decades. The population declines observed in such pinniped species as the harbor seal (*Phoca vitulina*), Steller sea lion (*Eumetopias jubatus*), and northern fur seal (*Callorhinus ursinus*) (Pitcher 1990, Loughlin *et al.* 1992) are especially notable. For example, the number of harbor seals at Tugidak Island, south of Kodiak, once one of the largest harbor seal haul-out sites in the world, has decreased by 72-85% between 1976 and 1988 (Pitcher 1990). A similar reduction in Steller sea lions numbers in the Gulf of Alaska has forced the National Marine Fisheries Service (NMFS) to list this species as endangered under the Endangered Species Act.

In attempts to explain the observed pinniped declines, many hypotheses dealing with environmental and anthropogenic factors have been tested. Anthropogenic factors that could play a role in marine mammal declines include subsistence harvesting, exposure to pollutants, and human disturbance (Sease 1992, Lowry *et al.* 1996), although at this time these factors do not appear to be the primary cause for the widespread decline (Lowry *et al.* 1996). Few studies also have examined the impacts of fishery interactions and environmental oscillations on animal condition in Alaskan pinniped populations (Fadely and Castellini 1996, Fadely 1997, Pitcher *et al.* 2000). While there appears to be evidence that suggests that some pinniped populations, such as harbor seals in Prince William Sound, are exposed to some physical, physiological, or environmental stress (Zenteno-Savin *et al.* 1997), the cause has not been established.

Nutritional limitation as a result of changes in prey distribution or quality has been proposed as the hypothesis to explain the decline of the harbor seal throughout parts of Alaska. These nutritional limitations, which may be a function of a switch in the prey base from a more lipid rich herring to a less fatty pollock, may affect nutritional status of an animal and promote changes in body size or composition (Harder and Kirkpatrick 1994). Historically, primary prey items of the harbor seals in Alaska have been pollock (*Theragra chalcogramma*), octopus (*Octopus* sp.), capelin (*Mallotus villosus*), eulachon (*Thaleichthys pacificus*), and herring (*Clupea pallasii*, Pitcher 1980). Studies using fatty acid signatures to determine the diet of harbor seals in the Gulf of Alaska have indicated that large pollock remain a primary prey item (Iverson *et al.* 1997), although the relative importance of this species could not be determined. Shifts in prey abundance or prey quality are known to cause physiological stress to individual animals, which can be detected by morphological or physiological measurements (Castellini and Calkins 1993, Calkins *et al.* 1998) and hence potentially used as an indicator of health in a population (Hanks 1981).

Before modeling nutritional health in free-ranging harbor seals it is important to clarify and characterize the condition of a population through the measurements of individual animals. Hanks (1981), describing five methods to quantify demographic vigor, concluded that in a large vertebrate population one can measure the condition of an individual and extrapolate to the population by estimating deposited fat reserves, adrenocorticol hypertrophy, blood chemistry and hematology, urine hydroxypoline, and/or some body mass index. Harder and Kirkpatrick (1994) subsequently added that to assess a nutritional index in terrestrial mammals estimates of whole body fat (BIA, D_2O), kidney fat index, marrow fat, and blood and urine characteristics are needed. For harbor seal pups within Prince William Sound and on Tugidak Island, Alaska, we used blood and morphometrics as an indicator of health because of the relative non-invasiveness of these techniques.

Numerous blood characteristics have been investigated and used to determine nutritional status of wild animals, however, clear interpretation of the results remain difficult. The most important aspect of using blood parameters is to identify boundary conditions or sources of variation. Problems arise when trying to rank blood boundary conditions in order of importance because parameters are unique and often correlated. Some early work in population health assessment used blood data for gross nutritional status, but as stated by Franzmann (1985), researchers should combine blood chemistry analysis with morphometrics, behavior and population statistics and standardize the analysis. Fadely (1997) provided excellent results of blood chemistry and hematology combined with morphometric measurements of adult harbor seals in Alaska. His approach of using a binomial model to determine the extent of individual outliers provided possible clues to population condition. Since blood variables are not independent, use of this model would create an additive effect for "outliers". However, this method combined with discriminate analysis (hierarchical analysis) may provide a better estimator of "clinical" significance for individuals within the population and thus provide an important link to the nutritional status (e.g. "junk food" hypothesis) of a wild seal population. The underlying assumption of the "junk food" hypothesis is that shifts in prey quality may decrease the nutritional efficiency of a predator, causing physiological and morphological stress. Prior to this study, no nutritional/physiological studies have been conducted on harbor seals in Alaskan waters that directly test the "junk food" hypothesis. We propose to develop reference ranges for harbor seal pups in Alaskan waters from two distinct populations and determine if using outlier methods can assess clinical significance.

METHODS

Field Techniques

Within PWS, harbor seals were live-captured by net entanglement using methods previously described by Frost *et al.* (1995). Once captured, the seals were transported to shore or ship, weighed with an electronic hanging scale (Ohaus Model I-20W), and morphometric measurements gathered. Blood samples were drawn for laboratory analysis.

On Tugidak Island, researchers captured hauled out harbor seal pups usually at low tide during midday using large salmon nets or hoop nets. Once captured, the pups were manually restrained, weighed with an electronic hanging scale, morphometric measurements gathered and blood samples drawn for laboratory analysis.

In conjunction with this study, the Alaska Department of Fish and Game fitted harbor seals captured at Tugidak Island and within PWS with satellite-linked time-depth-recorders to determine dive behavior and movements.

Blood Chemistries

Blood samples were prepared in the field for shipment and ultimately transferred to the University of Alaska for further analysis. Plasma samples were sent to Fairbanks Memorial Hospital (FMH) for assessment of "standard" health indices and analyzed at our laboratory for indicators of dehydration, malnutrition and hormonal imbalance.

Blood from harbor seal pups at Tugidak Island and from PWS was drawn from the extradural vein using 3.5 inch 18 or 20 G spinal needles into various Vacutainer blood container tubes (heparinized, EDTA, and serum). Hematocrit was determined in the field using a microcrit centrifuge (Compur model 1100). Hemoglobin was determined in the field by pipetting 10 uL whole blood into 2.5 mL Drabkin's reagent for subsequent photometric determination, also a 1 mL sample of whole blood was frozen at -80C for water content determination. Remaining whole blood was centrifuged and the plasma separated and frozen at -80C. Blood smears were then made from EDTA tubes for differential counting. EDTA blood and blood smears were processed by FMH for complete blood counts (red and white blood cells and platelets), differential counts, and erythrocyte morphology. Approximately 1 mL heparinized plasma was sent to FMH for determination of standard plasma chemistries: sodium, potassium, chloride, calcium, phosphate, cholesterol, glucose, protein, blood urea nitrogen, albumin, creatinine, globulin, bilirubin, lactate dehydrogenase, alanine aminotransferase, aspartate aminotransferase, creatinine phosphokinase, gammaglobulin transferase, and alkaline phosphatase.

Morphometrics

Mass, girths at three locations (max, axial, and hip) along with standard length (SL; straight-line distance between tip of nose and tip of tail) were measured (± 1 cm) with the seal positioned dorsal side up. Blubber thickness was measured at a minimum of three locations (dorsally) at each girth measurement location using a portable ultrasonic unit (Scanoprobe II, Model 7310, Scanco, Inc.), similar to Gales and Burton (1987). Blubber thickness relative to body thickness was calculated by dividing the mean blubber thickness by the body radius at that girth site.

Statistics

Blood chemistry, hematology, and morphology parameters were analyzed to determine if any statistical differences were found for transformed data among regions and sexes for all harbor seal pups. Only non-hemolyzed plasma samples were used in the final statistical analysis. Lipemic samples were used if not statistically significant from non-lipemic samples. To calculate blood chemistry and hematology reference ranges for harbor seal pups, all values within any one blood parameter were pooled and calculated as $\pm$ 2 standard deviations.

Outlier determination

Plasma chemistry data from all pups sampled during 1997 through 1999 were screened for outliers based on hierarchical clustering analysis (SYSTAT, v. 9.0). Interpretation of an outlier (goodness of fit) using hierarchical clustering analysis is based on Euclidean distances or Pearson Correlations (Manly 1994). Each individual harbor seal pup deemed an outlier in the population was examined for the specific blood parameter that created its outlier status, using hierarchical cluster analysis against minimum and maximum blood values.

Plasma chemistry data from all pups sampled from 1997-1999 were also screened for outliers based in calculated reference range criteria (Fadely 1997). Expected frequencies of numbers of outliers per seal were calculated from a binomial expansion of $(p+q)^k$, where p is the probability of an outlier (0.05) and q was the probability of no outlier (0.95), and k is the number of blood variables (21).

RESULTS

A total of 119 pups were captured from Tugidak Island (n=71) and within PWS (n=48) between 1997 and 1999. Significantly more males were captured on Tugidak Island (males=44, females=27) than within PWS (males=21, females =27). Normality for each blood parameter was determined by Kolmolgorov-Smirnoff Probability Test ($P<0.05$) along with Q-Q plots. Data were transformed to correct for non-normality. Alpha (α) levels were placed at 0.05 for all statistical tests.

Morphology

There was a significant difference in pup mass among regions, with PWS pups significantly heavier at time of capture for each year (PWS, mean =30.11 kg, SD = 5.8 kg; Tugidak, mean =26.97 kg, SD = 8.3 kg, P =0.004). Male pups were significantly heavier at time of capture than females regardless of region or year (P =0.004). Harbor seal pups captured at Tugidak were significantly longer (SL = 95.2cm) than seals captured within PWS (SL = 92.8cm, P =0.03).

Absolute blubber thickness was significantly greater for pups captured within PWS when compared to seal pups from Tugidak (ANOVA; mid, $P<0.001$; ax, P=0.027; hip, $P<0.001$). Relative mean blubber thickness was also greater for pups within PWS (Fig 1., P=0.007); however, there was no significant difference among years (P=0.862) or gender (P=0.881).

Correlation of absolute morphometric measurements were highly variable for pooled harbor seal pups from within PWS (Table 1) and Tugidak (Table 2). When pooling all seal pups, the highest positive correlation was seen between maximum girth and body mass (Pearson correlation coefficient, r = 0.84). A least squares linear regression revealed: Mass (kg) = 0.672 (max girth) - 28.16; Max Girth (cm) = 0.996 (mass) + 55.16, for all pups sampled in Alaskan waters (N =119, r^2 = 0.70).

Hematology

Hematology values for pups from Tugidak revealed significantly higher hemoglobin and MCHC levels than for pups captured within PWS (Table 3). Hemoglobin and MCHC values between years were also significantly different (Hb, P=0.006, Tukey test revealed 1999 was significantly lower than 1997; MCHC, $P<0.001$). Hematocrit values were significantly greater for pups caught within PWS than for Tugidak pups (p=0.005). Interestingly, monocyte and eosinophil levels were also higher in PWS pups when compared to pups captured on Tugidak (Table 3).

Blood chemistry

Except for ALT and alkaline phosphatase, blood chemistry values from lipemic samples were not significantly different from non-lipemic samples and thus were used in all statistical comparisons (PWS, N =48; Tugidak, N =61). (you say this in the methods under the heading "Statistics").

Nine blood chemistry values (43%) were statistically different for harbor seal pups between Tugidak and PWS (Table 4). Comparison among locations revealed significantly different blood chemistry values for AP, ALT, AST, BUN, BUN-Creatinine, phosphorus, Na, K, CPK (Table 4).

Outliers

Hierarchical cluster analysis of blood chemistry parameters for harbor seal pups on Tugidak yielded nine (13%) individual outliers, whereas none were detected in the PWS population (Figs. 2 and 3). Outliers were more prevalent in pups sampled in 1997 than those sampled in other years (Figs. 4a-i). Using hierarchical cluster analysis, it was possible to isolate the blood parameters that were responsible for creating outlier status for individual pups, however, no trends were evident (Fig. 5).

There was general agreement between the hierarchical cluster analysis and the binomial expansion model for gross outlier determination. However, the binomial model indicated two individual outliers (with excess of 6 blood parameters responsible for the outlier status) for Tugidak and no outliers in the PWS population (Figs. 6a-b).

DISCUSSION

In this study we witnessed significantly lower body mass and relative body fat along with longer body lengths in harbor seal pups captured on Tugidak Island when compared to pups within PWS. As has been suggested by various authors, body fat changes are positively correlated with changes in body mass (Rosen and Renouf 1995, Markussen *et al.* 1994, Ryg *et al.* 1990). Trites and Bigg (1992) revealed that body length and mass along with condition of the northern fur seals fluctuated due to the availability of prey. Calkins *et al.* (1998) suggested that Steller sea lion body size mirrored prey availability (or change in available prey items). It was uncertain, however, if declines in body size in these species were a function of a density dependent factors, such as reduced carrying capacity (Trites and Bigg 1992, Calkins *et al.* 1998). As pointed out by Mrosovsky (1976), body weight cycle is a mirror of the environment, reflecting the seasonal alternation between abundance and scarcity of prey. The nutritional environment available to harbor seal pups during the lactation period is almost exclusively from its mother and thus any environmental stress placed upon the mother should also be witnessed in the pup. In our study, differences in mass between populations is possibly a function of development, however, the differences in relative body fat in pups between locations may be a symptom of nutritional stress, with the pups on Tugidak more "unhealthy" from a statistical standpoint. Whereas we captured pups from each location during identical sampling periods (25 June- 2 July), pupping dates may have differed by as much as a week that could produce significant differences in morphological and certain hematological parameters (J.

Burns pers. comm.). When compared to data collected in the 1960's, the onset of pupping on Tugidak has steadily increased over the past decade (Jemison 1997). Discussions concerning the causality of delayed pupping include nutrition problems faced by adult females prior to lactation (Jemison 1997).

In general, female marine mammals seem to follow a pattern of fattening preceding anorexia for the period leading to and then following pupping (Mrosovsky 1976). For harbor seals, it is widely accepted that prior to reproduction the mass of the seal increases which would prove important, not only energetically during the fasting period, but also for parental care, especially during the short lactation period in seals when pups need to gain weight rapidly. The potential for additional stress during environmental or nutritional perturbations may be magnified since harbor seals have a very low insulation quality relative to other marine mammals (Robbins 1993). It has been reported that harbor seals are fast-adapted (Castellini and Rea 1992), however, these phocids differ from other fasting mammals because they remain active throughout their periods of food deprivation, which may cause rapid changes in body size. The length of a fast is usually proportional to the extent of lipid reserves (Cherel *et al.* 1993, Atkinson *et al.* 1996) and during an intense lactation period, harbor seal females not only have to maintain homeostasis during a relatively short fasting, but also need to offset the losses of water through milk, urine and evaporation (Schweigert *et al.* 1993). Female harbor seals overcome problems regarding food and water limitations during lactation by foraging (Boness *et al.* 1994); however, this foraging would lead to a reduction in parental care and thus increase the potential for relatively "unhealthy" pups.

In addition to maternal investment advantages, the digestive physiological benefits of increased nutritional prey items for harbor seals prior to lactation are numerous. A high fat, high-energy diet may have a greater assimilation efficiency, which may increase passage rate and therefore emptying time. Markessen *et al.* (1994) found that harbor seals on a herring diet (high fat) had a lower heat increment of feeding thus a lower metabolic rate. On a low energy diet (i.e., pollock) less energy would be absorbed and coupled with an increased retention time (Trumble and Castellini unpub data) creating a need for a greater intake to meet energetic demands. It has been suggested that some pinnipeds, such as the California sea lion, can adjust their assimilation efficiencies to accommodate prey quality (Fadely *et al.* 1994). However, this has not been reported for harbor seals. Pritchard and Robbins (1990) reported that the digestive and metabolic efficiencies for some mammals on various qualities of diets were not significantly different.

Of the studies presenting hematological reference ranges for harbor seals, this is the first study to compare harbor seal pups from various geographic locations. Preliminary screening of blood panels based on calculated reference ranges did not present indications of population-level chronic diseases consistent with findings from serological survey data for common phocid diseases (Frost *et al.* 1995). Without histological determinations of disease-state, unhealthy seals may have been included in our reference ranges. Harbor seal pups captured from Tugidak Island and PWS revealed gender-specific differences, with greater Hb levels in female compared to male pups on Tugidak Island. Knick *et al.* (1993) assessed nutritional status of bobcats occupying high, medium, or low prey density areas and found that serum insulin and cholesterol declined significantly with prey densities, but RBC counts, Hb, and Hct represented the best index of change in nutritional status. While Franzmann and Schwartz (1988) also recommended using Hb and Hct to assess condition, we however, have not determined any correlation between Hb and Hct for pups captured between locations. While low Hb concentration may indicate anemia, elevated levels may suggest dehydration; however, it is important to acknowledge that these levels may be a function of sample size and/or development of the pup. deSwart *et al.* (1995) fed 22 harbor seals contaminated herring for 125 weeks and found significant differences in Hct when fed the contaminated fish. No significant differences were found in other hematological parameters. However, they did suggest that Hb, MCH, MCHC, MCV all declined with age and development. Thompson *et al.* (1997) stated that RBC counts and MCHC declined during periods of "bad" prey years, but also mentioned that these parameter changes may have been a function of age. Due to logistic constraints we were unable to perform RBC counts for pups captured on Tugidak.

There were also gender-specific differences in monocyte levels on Tugidak (Table 1). These data may suggest some inflammatory response or impaired immune function, although at this time it would be difficult to ascertain without further tests. It is important to note that none of these differences indicate diseased seals, as these values were all within the normal reference ranges.

Many blood chemistry characteristics have daily rhythms (Rosen and Renouf 1995, deSwart *et al.* 1995), and may change as a function of season (Ryg *et al.* 1990, DelGuidice *et al.* 1992, Knick *et al.* 1993), age (Burns *et al.* 1998), gender (Heidel *et al.* 1996) as well as diet, molt, lactation, or environmental perturbations. Of the blood chemistry variables tested during this study, many blood electrolytes, blood proteins and liver enzymes were varied significantly among regions (Table 2). Tugidak Island pups had significantly greater sodium levels than pups captured within PWS, which may indicate dehydration in Tugidak pups because sodium levels fluctuate with hydration state of mammals (DelGiudice *et al.* 1987). While electrolytes may, in the case of pups, be linked to the nutritional state of the mother (Schweigert 1993) we have no evidence to make this connection. Further tests are being done on the hydration state of the pups.

Liver enzymes, which were also elevated when compare to PWS pups, are known to become elevated during periods of stress such as capture. Interestingly, we noticed that liver enzymes (ALT, GGT) were more prevalent than other blood parameters responsible for individual pup outliers (Fig 5). Based on captive studies of harbor seals fed pollock and herring (Trumble *et al.* unpub data) both GGT and ALT were affected by diet.

In this study, harbor seal pups from Tugidak had significantly higher BUN values than pups from PWS. LeReche (1974) used BUN (or SUN) as an indicator of nutritional status, and it has become one of the most widely used indices because it is unaffected by capture stress, handling or drugs. Knick *et al.* (1993) reported a very large increase in SUN and triglycerides in emaciated animals (catabolizing fat and protein reserves). While we noticed elevated levels in Tugidak pups, we did not observe any signs of emaciation during any sampling period. We believe that these differences are largely due to differences in development.

Outliers

Examination of the statistical outliers of blood chemistry using both cluster analysis and binomial expansion methods reveal a greater number of outliers for the Tugidak harbor seal pup population. Hierarchical cluster analysis appeared to be more sensitive, revealing nine outliers (13%) compared to two for binomial expansion methods. A benefit of cluster analysis is its ability to identify the blood parameter(s) responsible for the outlier status of an animal. Blood parameter trends can be subsequently assessed for the population and determined if there are indications of a clinical significance to health.

Development of reference ranges appropriate for free-ranging Gulf of Alaska harbor seal pups permits examination of veterinary blood panels with more confidence than would have been possible utilizing ranges published with adult values, small sample sizes, or from captive or free-ranging seals of other geographic regions. We must stress that, especially for pups, there appears to be significant differences in several blood parameter reference ranges and thus if using outlier methods to assess health, a reference range for each population must be established. The assumption of setting a normal reference range within two standard deviations is that outliers will be mostly comprised of potentially physiologically compromised animals, although this may not hold true (Kerr 1989).

These data will be further analyzed to determine the condition of health and the potential health differences (outliers) between harbor seals within PWS and on Tugidak. It should be stressed that "statistically significant" does not necessarily correspond to "clinically significant", or that the animals are clinically unhealthy. Ongoing studies related to this project will provide valuable information regarding the energetic role of primary forage species in Alaskan waters along with seasonal or dietary variations of radically different prey items.

CONCLUSION

Harbor seal pups from Tugidak Island and PWS differed significantly in several blood chemistry, morphological, and hematology parameters. However, we must use caution in labeling one population less "healthy" based on differences in these variables alone. The use of hierarchical cluster analysis to detect outliers and their determinants has provided us a technique to further assess the health of pinniped populations. This technique along with other field health assessment methods (e.g. D_2O, BIA) will provide researchers with the ability to discern health, and in the case of pups, differences due to development, in free-ranging phocids.

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LITERATURE CITED

Atkinson, S. N., R. A. Nelson, and M. A. Ramsey. 1996. Changes in the body composition of fasting polar bears (Ursus maritimus): The effect of relative fatness on protein conservation. Phys. Zool. 69(2):304-316.

Boness, D. J., W. D. Bowen, and O. T. Oftedal. 1994. Evidence from time-depth recorders of a foraging cycle during lactation in a small phocid, a harbor seal. Behav. Ecol. Sociobiol.

Burns, J. M., S. J. Trumble, M. A. Castellini, and J. W. Testa. 1998. The diet of Weddell seals in McMurdo Sound, Antarctica as determined from scat collections and stable isotope analysis. Polar Biology 19:272-282.

Calkins, D.G., E. F. Becker, and K. W. Pitcher. 1998. Reduced body size of female Steller sea lions from a declining population in the Gulf of Alaska. Marine Mammal Science 14(2):232-244.

Castellini, M. and D. Calkins. 1993. Mass estimates using body morphology in Steller sea lions. Marine Mammal Science 9:48-54.

Castellini, M. .A. and L.D. Rea. 1992. The biochemistry of natural fasting at its limits. Experientia 48:575-582.

DelGiudice, G. D., L D. Mech, K. E. Kunkel, E. M. Gese and U. S. Seal. 1992. Seasonal patterns of weight, hematology, and serum characteristics of free-ranging female white-tailed deer in Minnesota. Can. J. Zool. 70:974-983.

deSwart, R. L., P. S. Ross, L. J. Vedder, F. B. T. J. Bionk, P. J. H. Reijnders, P. G. H. Mulder and A. D. M. E. Osterhaus. 1995. Haematology and clinical chemistry values for harbour seals (Phoca vitulina) fed environmentally contaminated herring remain within normal ranges. Can. J. Zool. 73:2035-2043.

Fadely, B. F., and M. A. Castellini. 1996. Hematology and plasma chemistry values for the Gulf of Alaska harbor seals, and preliminary regional comparisons 1993-1995. In: Harbor seal investigations in Alaska, Annual Report 1996. Alaska Dept. Fish and Game. NOAA Grant NA57FX0367.

Fadely, B. S. 1997. Inverstigations of harbor seal (Phoca vitulina) health status and body condition in the Gulf of Alaska. Ph.D. Dissertation Univ. Alaska Fairbanks. 183 pp.

Fadely, B.S., J.M. Castellini and M A. Castellini. 1998. Recovery of harbor seals from EVOS: condition and health status. *Exxon Valdez* Oil Spill Restoration Project Annual Report (Restoration Project 96001), University of Alaska Fairbanks, Alaska. 53pp.

Franzmann A.W. 1985. Assessment of nutritional status. In: Bioenergetics of wild herbivores. R. J. Hudson and R. G. White eds. CRC press. Pp 239-259.

Franzmann, A. W. and C. C. Schwartz. Evaluating condition of Alaskan black bears with blood profiles. J. Wildl. Manage. 52(1):63-70.

Frost, K. J., L. F. Lowry and J. Ver Hoef. 1995. Habitat use, behavior and monitoring of harbor seals in Prince William Sound, Alaska. Annual Rep. For *Exxon Valdez* Oil Spill Restoration Project Restoration Projects 94064 and 94320-F), Alaska Dept. of Fish and Game, Wildlife Conservation Division, Fairbanks. 87pp.

Hanks, J. 1981. Characterization of population condition. In: Dynamics of large mammal populations. Fowler, C. W. and T. D. Smith, eds. Pp. 47-73.

Harder, J. D. and R. L. Kirkpartrick. 1994. Physiological methods in wildlife research. In: Research and management techniques for wildlife habitats. T. A. Bookhout ed., Pp. 275-306.

Heidel, J. R., L. M. Philo, T. F. Albert, C. B. Andreasen, and B.V. Stang. 1996. Serum chemistry of bowhead whales (Balaena mysticetus). J. Wildl. Diseases. 32(1):75-79.

Iverson, S. J., K. J. Frost, L. F. Lowry. 1997. Fatty acids signatures reveal fine scale structure of foraging distribution of harbor seals and their prey in Prince William Sound, Alaska. Marine Ecology Progress Series. 151:255-271.

Kerr, M. G. 1989. Veterinary laboratory medicine. Clinical biochemistry and hematology. Blackwell Scientific Publ., Oxford. 270pp.

Knick, S. T., Hellgren, E. C. and U. S. Seal. 1993. Hematologic, biochemical, and endocrine characteristics of bobcats during a prey decline in southeastern Idaho. Can. J. Zool. 71:1448-1453.

Loughlin, T. R., A. S. Perlov, and V. A. Vladimirov. 1992. Range-wide survey and estimation of total numbers of Steller sea lions in 1989. Mar. Mamm. Sci. 8:220-239.

Lowry, L. F., R. L. Zarnke, and J. P. Lewis. 1996. Disease studies of Alaskan harbor seals. In: Harbor seal investigations in Alaska, Annual Report 1996. Alaska Dept. Fish and Game. NOAA Grant NA57FX0367.

Manly, B. F. J. 1994. Multivariate statistical methods, a primer. Chapman and Hall, London.

Martensson, P E., E. S. Nordoy, and A. S. Blix. 1994. Digestibility of crustaceans and capelin in harp seals (*Phoca groenlandica*). Mar. Mam. Sci. 10(3):325-331.

Merrick, R. L., R. Brown, D. G. Calkins and T. R. Loughlin. 1995. A comparison of Steller sea lion pup masses with increasing and decreasing populations. Fish. Bull. U.S. 93:752-757.

Mrosovsky, 1990. Rheostasis: The physiology of change. Oxford Univ Press. 174 pp.

Pitcher, K. W. 1980. Food habits of the harbor seal (Phoca vitulina richardsi) in the Gulf of Alaska. Fish. Bull. 78(2):544-549.

Pitcher, K. W. 1990. Major decline in the number of harbor seals (Phoca vitulina richardsi) on Tugidak Island, Gulf of Alaska. Mar. Mamm. Sci 6(2):121-134.

Pitcher, K. W., D. G. Calkins, and G. W. Pendleton. 2000. Steller sea lion condition indices. Mar. Mamm. Sci. 16(2):427-436.

Pritchard, G. T. and C. T. Robbins. 1990. Digestive and metabolic efficiencies of grizzly and black bears. Can. J. Zool. 68:1645-1651.

Robbins, C. T. 1983. Wildlife nutrition and feeding. Academic Press, New York. 352 pp.

Rosen, D. and D. Renouf. 1995. Variation in the metabolic rates of captive harbour seals. Pp. 393-399 in: Blix, A. S., Walloe, L. and O. Ulltang, eds. Whales, seals and man. Elsevier Science. B. V.

Ryg, M., T. G. Smith, and N. A. Oritsland. 1990. Seasonal changes in body mass and body composition of ringed seals (Phoca hispida) on Svalbard. Can. J. Zool. 68:470-475.

Schweigert, F. J. 1993. Effects of fasting and lactation on blood chemistry and urine composition in the grey seal (Halichorus grypus). Comp. Biochem, Physiol. 105A(2):353-357.

Sease, J. L., 1992. Status review: Harbor seals in Alaska. NMFS AFSC Processed Rep. 92-15. 74pp.

Thompson, P. M., D. J. Tollit, H. M. Corpe, R. J. Reid, and H. M. Ross. 1997. Changes in haematological parameters in relation to prey switching in a wild population of harbour seals. Func. Ecol. 11(6):743-750.

Trites, A. W. and M. A. Bigg. 1992. Changes in body growth of northern fur seals from 1958 to 1974: density effects or changes in the ecosystem? In: Fisheries Oceanography, v. 1 no. 2. 127-136.

Zenteno-Savin, T., M. A. Castellini, L. D. Rea, and B. S. Fadely. 1997. Plasma haptoglobin levels in threatened Alaskan pinniped populations. J. Wildl. Diseases. 33(1):64-71.

Table 1. Pearson correlation coefficients for morphometric measurements taken from PWS harbor seal pups between 1997 and 1999. N =number sampled, G = girth, and B = Blubber.

	Mass	SL	Ax Girth	Hip Girth	Max Girth	Blubber Ax	Blubber Max	Blubber Hip
N	58	58	58	51	57	39	39	39
Mean	29.2	93.6	83.5	68.3	84.2	23.9	24.9	25.7
Mass	1							
SL	0.427	1						
Ax G	0.622	0.214	1					
Hip G	0.555	0.287	0.352	1				
Max G	0.742	0.255	0.737	0.528	1			
B Axial	0.331	0.419	0.331	0.143	0.275	1		
B Max	0.272	-0.05	0.211	0.131	0.254	0.429	1	
B Hip	0.299	0.172	0.369	0.336	0.393	0.41	0.353	1

Table 2. Pearson correlation coefficients for morphometric measurements taken from Tugidak Island harbor seal pups between 1997 and 1999. N =number sampled, G = girth, and B = Blubber.

	Mass	SL	Ax Girth	Hip Girth	Max Girth	Blubber Ax	Blubber Max	Blubber Hip
N	71	71	71	24	69	39	45	45
Mean	26.9	95.4	81.8	67	82.8	21.2	21.5	21.3
Mass	1							
SL	0.536	1						
Ax G	0.439	0.44	1					
Hip G	0.681	0.321	0.419	1				
Max G	0.898	0.46	0.546	0.685	1			
B Axial	0.13	0.162	-0.077	0.17	0.088	1		
B Max	0.797	0.403	0.303	0.573	0.649	0.404	1	
B Hip	0.398	0.252	0.721	0.289	0.434	0.091	0.381	1

Table 3. Harbor seal pup reference ranges for hematology values and differential leukocytes counts collected at Tugidak Island and within Prince William Sound 1997-1999. P values listed among regions; reference range calculated as $\pm$ 2SD.

Variable	*PWS mean*	*PWS SD*	*PWS CV*	*n*	*TUG mean*	*TUG SD*	*TUG CV*	*n*	*P value among regions*	*Total means* *Mean*	*SD*	*CV*	*Reference Range PWS*	*Reference Range TUG*	*Reference Range Total*
Hematocrit [a]	0.559	0.033	0.06	53	0.542	0.037	0.07	69	**0.005**	0.549	0.037	0.07	0.493-0.625	0.468-0.616	0.475-0.623
Hemoglobin(g/dL) [b]	23.52	1.77	0.07	53	21.16	1.81	0.09	67	**0.000**	22.0	2.14	0.09	19.98-27.06	17.54-24.78	17.72-26.28
MCHC (g/L)	41.89	2.61	0.06	53	39.07	3.08	0.08	67	**0.000**	40.32	3.2	0.08	36.67-47.11	32.91-45.23	33.92-46.72
PMN	56.54	12.5	0.22	52	60.52	8.5	0.14	63	0.081	58.7	10.65	0.18	31.54-81.54	43.52-77.52	37.4-80.0
Lymphocytes	29.08	9.41	0.32	52	27.56	10.4	0.38	63	0.559	30.09	9.97	0.35	10.26-47.9	6.76-48.36	10.15-50.03
Monocytes [c]	11.44	4.87	0.49	*45*	9.1	5.74	0.63	59	**0.043**	9.5	5.37	0.57	1.7-21.18	0.0-20.58	0.0-20.24
Eosinophils	3.98	3.65	0.92	41	2.12	1.6	0.76	50	**0.006**	2.96	2.87	0.97	0.0-11.28	0.0-5.32	0.0-8.7
Bands	2.5	1.61	0.65	30	1.3	0.5	0.4	7	INS	2.27	1.54	0.68	1.0-5.72	0.3-2.3	0.0-5.35
Basophils [e]	3.0	-----	1.0	1	0.35	0.67	1.9	29	INS	0.43	0.82	1.9		0.0-1.69	0.0-2.07
NRBC [d]	2.42	1.08	0.448	12	2.83	3.9	2.5	40	0.469	1.77	3.49	1.97	0.26-4.58	0.0-10.63	0.0-8.75

[a] Statistics performed on arcsine transformed data.
[b] Statistics performed on log transformed data.
[c] Statistics calculated from square root transformed data.
[d] Non-normal distribution (Q-Q plot, Kolmolgorov-Smirnoff Probability Test: $p<0.05$), statistics calculated using non-parametric tests
[e] Sample size insufficient for statistical analysis (INS)

Table 4. Harbor seal pup blood chemistry values for Prince William Sound and Tugidak Island, Alaska from 1997-1999. Reference ranges are ± 2SD; N =40 for PWS and n=59 for Tugidak Island unless noted otherwise.

Variable	*PWS Mean*	*PWS SD*	*PWS CV*	*TUG Mean*	*TUG SD*	*TUG CV*	*P value*	*Total mean values PWS+TUG*			*Reference Range PWS*	*Reference Range TUG*	*Reference Range Total*
								Mean	*SD*	*CV*			
Sodium (mmol/L) [a d]	144.2	2.18	0.015	148.4	10.4	0.07	**0.011**	146.7	8.4	0.06	139.8-148.6	127.6-169.2	129.9-163.5
Potassium (mmol/L) [b c e]	3.57	0.21	0.07	3.88	0.41	0.10	**0.000**	3.76	0.38	0.1	3.15-3.99	3.1-4.7	3.0-4.52
Chloride (mmol/L)	104.9	3.06	0.03	105.07	13.53	0.13	0.948	105	10.5	0.1	98.8-111.0	78.0-132.1	84-126
Glucose (mg/dL)[e]	162.8	20.05	0.12	168.1	29.07	0.17	0.606	165.9	25.8	0.16	122.7-202.9	109.9-226.2	114.-217.5
Phosphorus (mg/dL)	5.96	1.26	0.21	7.59	1.23	0.16	**0.000**	6.93	1.48	0.21	3.44-8.48	5.1-10.0	3.97-9.89
Calcium (mg/dL)[c e]	9.23	1.59	0.17	10.03	1.61	0.16	0.097	9.75	1.63	0.16	6.0-12.4	6.8-13.2	6.49-13.0
BUN (mg/dL) [a]	31.1	6.38	0.20	38.3	8.05	0.21	**0.000**	35.37	8.2	0.23	18.34-43.9	22.2-54.4	18.9-51.8
Creatinine (mg/dL)	0.77	0.14	0.18	0.71	0.16	0.23	0.055	0.73	0.16	0.21	0.49-1.05	0.4-1.03	0.41-1.05
BUN:Creatine [d]	41.6	10.20	0.25	55.35	14.36	0.26	**0.000**	49.78	14.48	0.29	21.2-62.0	26.6-84.1	20.82-78.8
Cholesterol (mg/dL)	340.1	91.6	0.27	320.26	69.69	0.22	0.357	328.3	79.4	0.24	156.9-523.3	180.9-459.6	169.-487.1
Total Bilirubin (mg/dL)	0.48	0.19	0.41	0.74	1.13	1.52	0.175	0.64	0.89	1.39	0.1-0.86	0-3.0	0-2.42
Haptoglobin													
Total Protein (g/L) [a]	6.66	0.39	0.06	6.84	1.04	0.15	0.102	6.77	0.84	0.12	5.88-7.44	4.76-8.92	5.09-8.45
Globulin (g/L)	3.28	0.36	0.11	3.29	0.62	0.19	0.574	3.29	0.5	0.16	2.56-4.0	2.0-4.5	2.29-4.29
Albumin (g/L)	33.7	1.78	0.05	34.88	5.33	0.15	0.066	34.4	4.29	0.12	30.1-37.3	24.2-45.5	25.8-43.0
Albumin:Globulin [d]	1.04	0.13	0.12	1.09	0.41	0.37	0.466	1.07	0.32	0.30	0.78-1.3	0.27-1.9	0.43-1.71
Alkaline Phosphatase [a]	292.5	171.1	0.58	428.03	185.8	0.43	**0.000**	373.3	191.2	0.51	0-634.7	56.4-800.0	0-755.7
AST (iu/L)	83.5	28.4	0.34	69.7	27.34	0.39	**0.031**	75.3	28.5	0.38	26.7-140.3	15.0-124.4	18.3-132.3
ALT (iu/L)	34.2	9.74	0.28	18.7	9.58	0.51	**0.000**	24.9	12.3	0.49	14.72-53.7	0-37.9	0.3-49.5
CPK (iu/L) [e]	856.8	700.9	0.818	437.8	296.85	0.68	**0.000**	608.8	540.5	0.9	0-2258.6	0-1031.5	0-1689.8
GGT (iu/L)	18.1	7.0	0.38	20.17	15.93	0.79	0.375	19.33	13.06	0.68	4.1-32.1	0-52.0	0-45.5
LDH (iu/L)	3463	836.4	0.24	3286	1480	0.45	0.619	3358	1258	0.37	1790-5135	326-6246	842-5874

[a] Statistics performed on log-transformed data
[b] Statistics performed on cube-root transformed data
[c] PWS n=38
[d] Data were arcsine transformed.

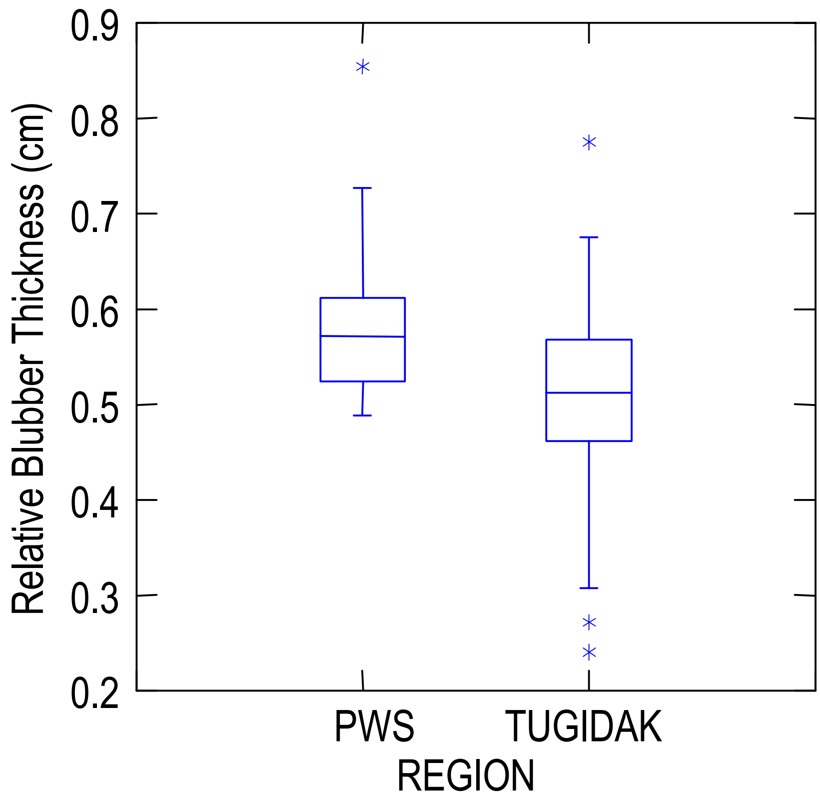


Figure 1. Relative blubber thickness for harbor seal pups captured on Tugidak Island and within PWS, Alaska 1997-1999.

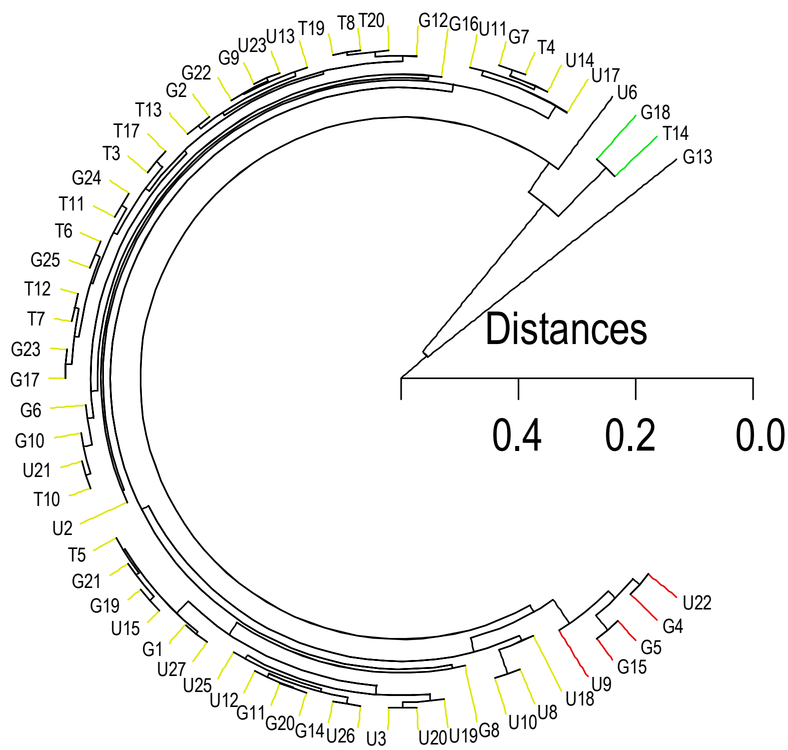


Figure 2. Hierarchical cluster analysis on blood chemistry for harbor seal pups captured on Tugidak Island, 1997-1999. Years labeled as T = 1997, U = 1998, G = 1999.

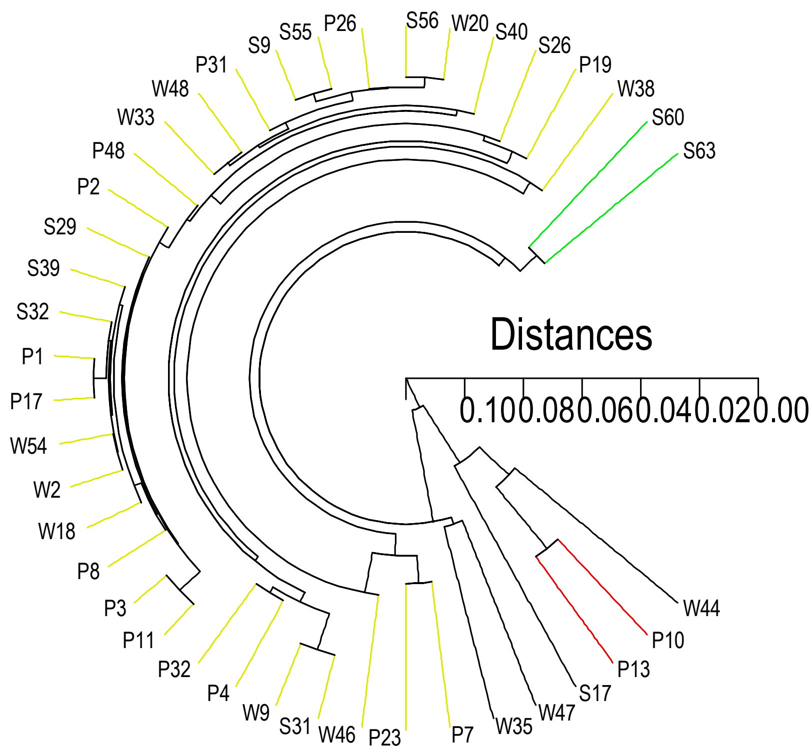


Figure 3. Hierarchical cluster analysis on blood chemistry for harbor seal pups captured within PWS, 1997-1999. Years labeled as P =1997, W = 1998, S = 1999.

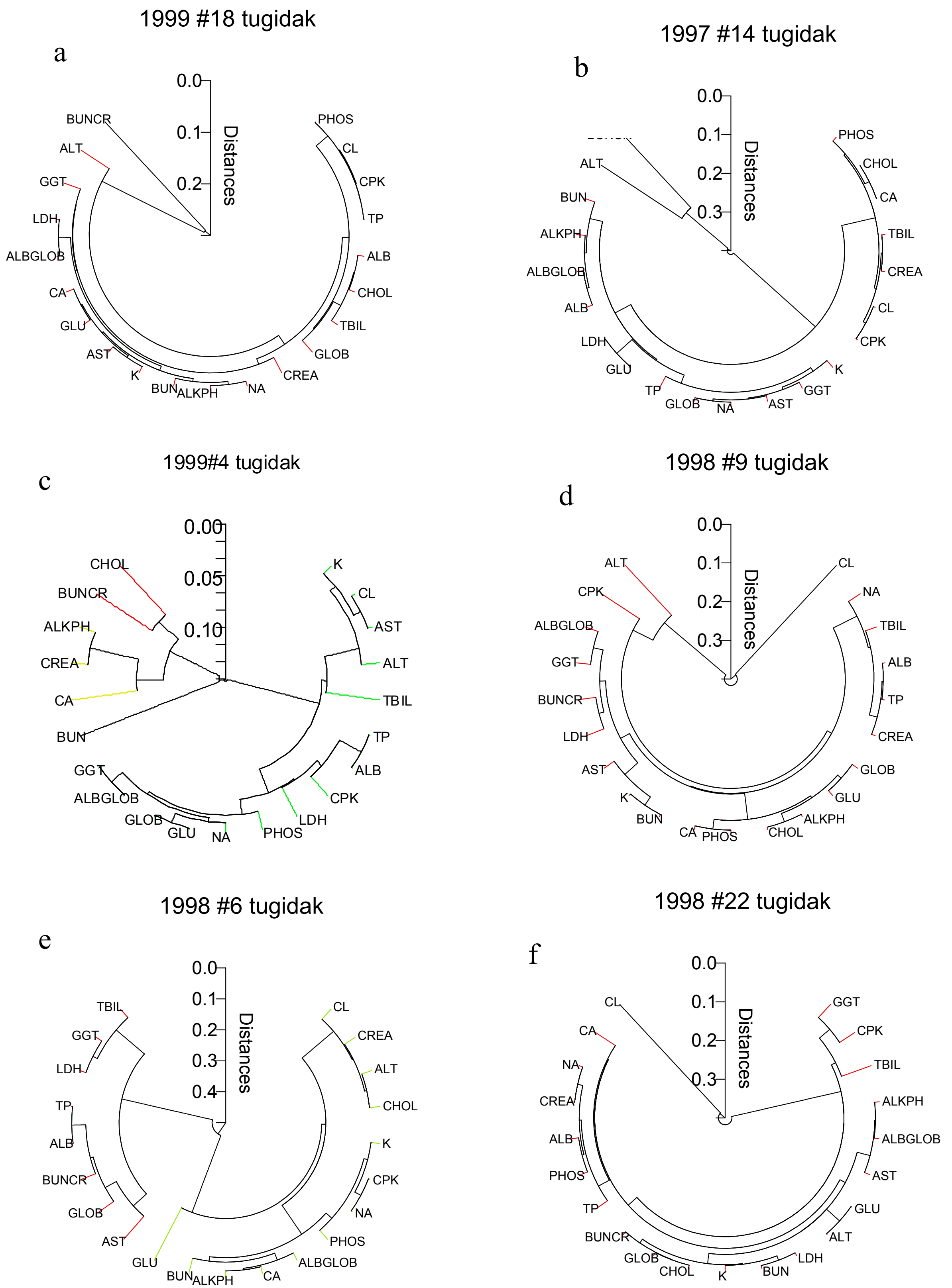


Fig 4a-f. Blood chemistry outliers for individual harbor seal pups captured on Tugidak Island, Alaska 1997-1999.

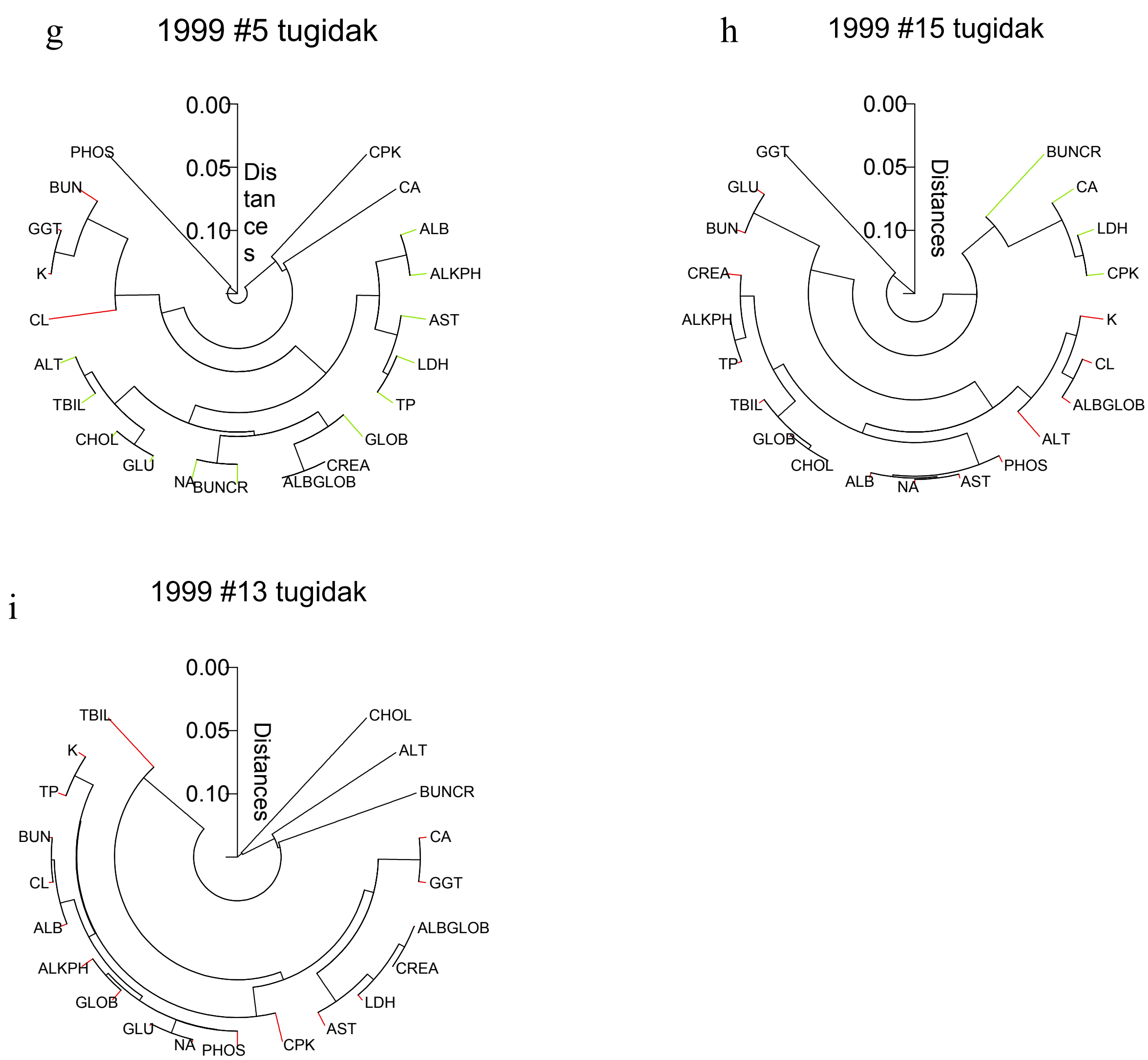


Figure 4g-i. Blood chemistry outliers for individual harbor seal pups captured on Tugidak Island, Alaska 1997-1999.

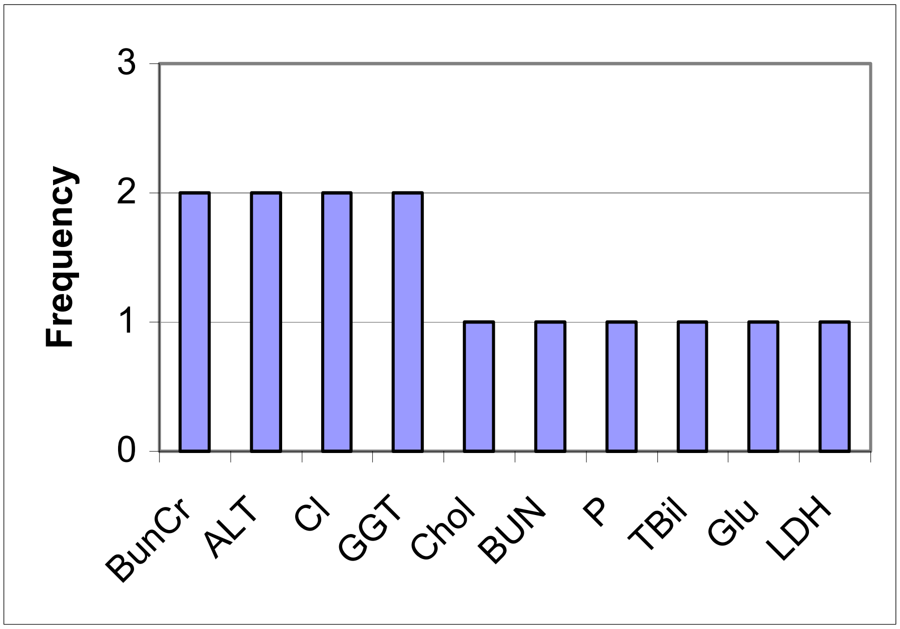
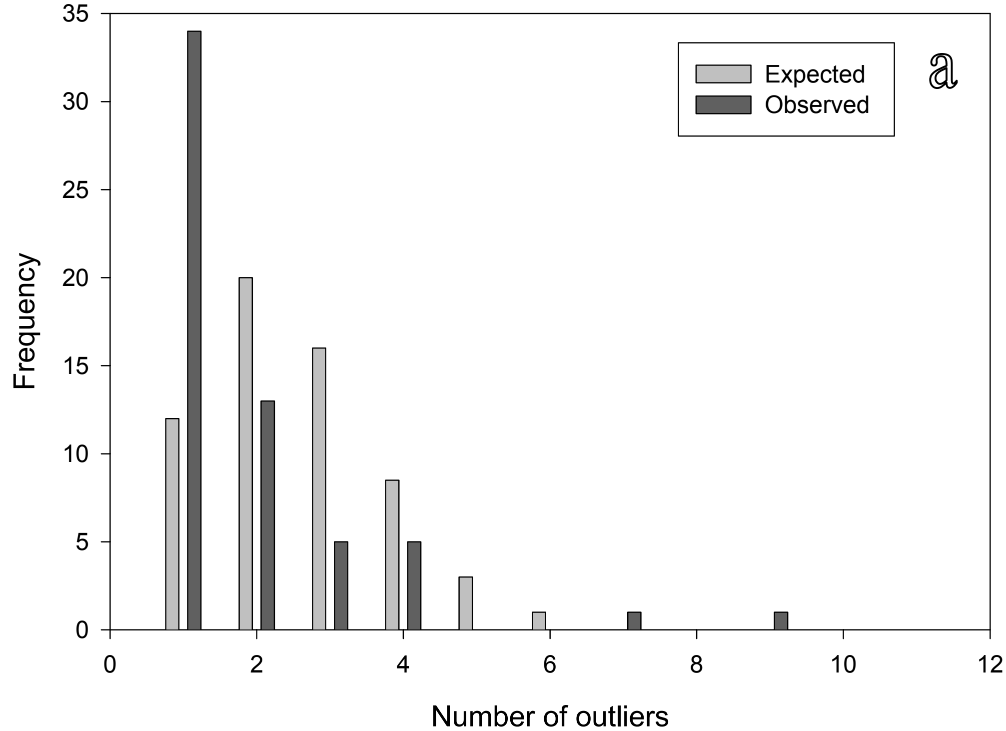


Figure 5. Frequency distribution of blood parameters from all outlier harbor seal pups from Tugidak Island, Alaska between 1997-1999.



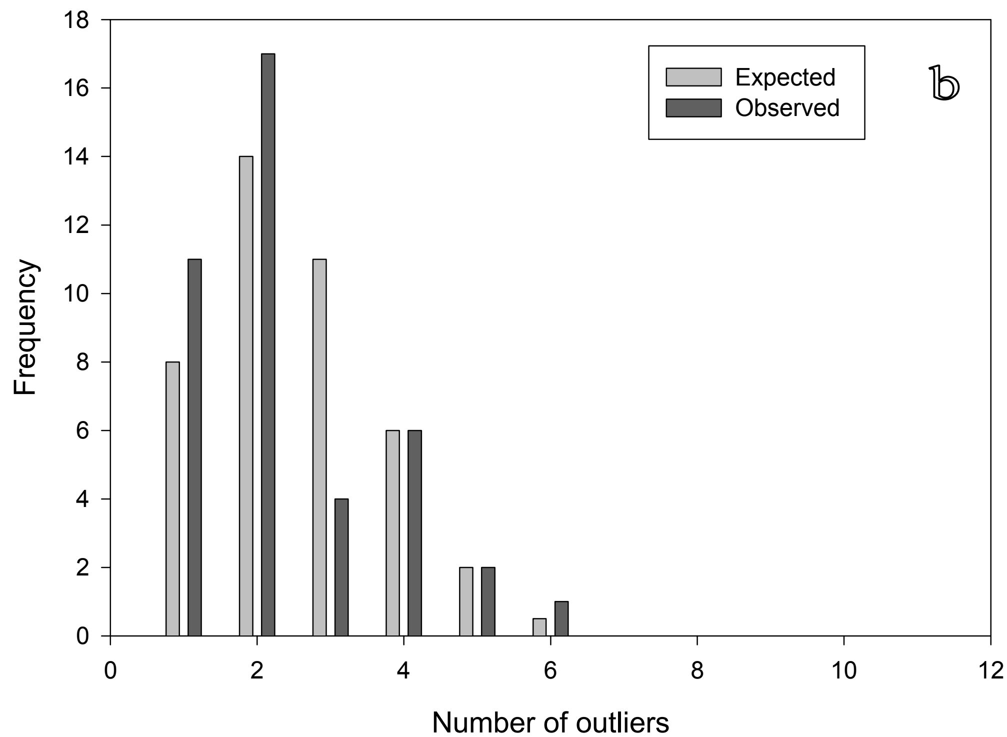


Figure 6a and b. Binomial distribution of expected versus observed outliers for blood parameters from harbor seal pups captured on Tugidak Island (a) and within PWS (b), 1997-1999.